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SPECIFIC NUCLEIC ACIDS

by

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**ANDROGEN REGULATED PROSTATE  
SPECIFIC NUCLEIC ACIDS**

**BACKGROUND OF THE INVENTION**

**FIELD OF THE INVENTION**

5           This invention relates generally to cancer and, more specifically, to prostate-specific genes that can be used to diagnose and treat prostate cancer.

**BACKGROUND INFORMATION**

10           Cancer is currently the second leading cause of mortality in the United States. However, it is estimated that by the year 2000 cancer will surpass heart disease and become the leading cause of death in the United States. Prostate cancer is the most common non-cutaneous  
15 cancer in the United States and the second leading cause of male cancer mortality.

          Cancerous tumors result when a cell escapes from its normal growth regulatory mechanisms and proliferates in an uncontrolled fashion. As a result of  
20 such uncontrolled proliferation, cancerous tumors usually invade neighboring tissues and spread by lymph or blood stream to create secondary or metastatic growths in other tissues. If untreated, cancerous tumors follow a fatal course. Prostate cancer, due to its slow growth profile,  
25 is an excellent candidate for early detection and therapeutic intervention.

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During the last decade, most advances in prostate cancer research have focused on prostate specific antigen (PSA), a member of the serine protease family that exhibits a prostate-specific expression profile. Serum PSA remains the most widely used tumor marker for monitoring prostate cancer, but its specificity is limited by a high frequency of falsely elevated values in men with benign prostatic hyperplasia (BPH). Other biomarkers of prostate cancer progression have proven to be of limited clinical use in recent surveys because they are not uniformly elevated in men with advanced prostate cancer. Due to the limitations of currently available biomarkers, the identification and characterization of prostate specific genes is essential to the development of more accurate diagnostic methods and therapeutic targets. In many cases, the clinical potential of novel tumor markers can be optimized by utilizing them in combination with other tumor markers in the development of diagnostic and treatment modalities.

Normal prostate tissue consists of three distinct non-stromal cell populations, luminal secretory cells, basal cells, and endocrine paracrine cells. Phenotypic similarities between normal luminal cells and prostate cancer cells, including the expression of PSA, have suggested that prostate adenocarcinomas derive from luminal cells. However, a number of recent studies suggest that at least some prostate cancers can arise from the transformation of basal cells and report the expression of various genes in normal prostate basal cells as well as in prostate carcinoma cells. These genes include prostate stem cell antigen (PSCA), c-met and Bcl-2. Because none of these genes is universally

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expressed in all basal cells and prostate carcinomas, the utility of these genes as diagnostic markers is limited. Likewise, because PSA is expressed in luminal secretory cells in normal prostate tissue, this antigen has limited utility as a marker for basal cell derived carcinomas.

Thus, there exists a need for the identification of additional prostate specific genes that can be used as diagnostic markers and therapeutic targets for prostate cancer. The present invention satisfies this need and provides related advantages as well.

#### **SUMMARY OF THE INVENTION**

The present invention provides androgen responsive prostate specific (ARP) nucleic acid and polypeptide molecules.

The present invention provides a substantially pure ARP1 nucleic acid molecule containing substantially the nucleotide sequence shown as SEQ ID NO:1. The invention also provides a substantially pure ARP1 nucleic acid molecule containing at least 10 contiguous nucleotides of nucleotides 722 to 1026 of SEQ ID NO:1. In one embodiment, the substantially pure ARP1 nucleic acid molecule includes at least 15 contiguous nucleotides of nucleotides 722 to 1026 of SEQ ID NO:1.

The present invention also provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from the individual with an ARP1 nucleic acid molecule that includes at least 10 contiguous nucleotides

of SEQ ID NO:1, determining a test expression level of  
ARP1 RNA in the sample, and comparing the test expression  
level to a non-neoplastic control expression level of  
ARP1 RNA, where an altered test expression level as  
5 compared to the control expression level indicates the  
presence of a prostate neoplastic condition in the  
individual. In one embodiment, the sample is prostate  
tissue. In another embodiment, the sample is blood,  
urine or semen. In yet another embodiment, the ARP1  
10 nucleic acid molecule has a length of 15 to 18  
nucleotides.

The present invention further provides a method  
for treating or reducing the severity of a prostate  
neoplastic condition in an individual by administering to  
15 the individual an ARP1 regulatory agent.

Further provided by the invention is a  
substantially pure ARP2 nucleic acid molecule containing  
substantially the nucleotide sequence shown as SEQ ID  
NO:2. The invention also provides a substantially pure  
20 ARP2 nucleic acid molecule that includes at least 10  
contiguous nucleotides of nucleotides 1128 to 4509 of SEQ  
ID NO:2. In one embodiment, such a substantially pure  
ARP2 nucleic acid molecule includes at least 15  
contiguous nucleotides of nucleotides 1128 to 4509 of SEQ  
25 ID NO:2.

The present invention additionally provides a  
method of diagnosing or predicting susceptibility to a  
prostate neoplastic condition in an individual by  
contacting a sample from the individual with an ARP2  
30 nucleic acid molecule that contains at least 10

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contiguous nucleotides of nucleotides 1128 to 4509 of SEQ ID NO:2, determining a test expression level of ARP2 RNA in the sample, and comparing the test expression level to a non-neoplastic control expression level of ARP2 RNA, 5 where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. A method of the invention can be practiced, for example, with a sample of prostate tissue, or a sample of blood, 10 urine or semen. An ARP2 nucleic acid molecule useful in a diagnostic method of the invention can be, for example, 15 to 18 nucleotides in length.

The present invention also provides a method for treating or reducing the severity of a prostate 15 neoplastic condition in an individual by administering to the individual an ARP2 regulatory agent.

The present invention additionally provides a substantially pure ARP3 nucleic acid molecule containing a nucleic acid sequence that encodes an ARP3 polypeptide 20 having at least 45% amino acid identity with SEQ ID NO:5. In one embodiment, the substantially pure ARP3 nucleic acid molecule contains a nucleic acid sequence encoding the amino acid sequence shown as SEQ ID NO:5. In another embodiment, the substantially pure ARP3 nucleic acid 25 molecule contains the nucleotide sequence shown as SEQ ID NO:4.

The present invention further provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a 30 sample from the individual with an ARP3 nucleic acid

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1 molecule containing at least 10 contiguous nucleotides of  
SEQ ID NO:4, determining a test expression level of ARP3  
RNA in the sample, and comparing the test expression  
level to a non-neoplastic control expression level of  
5 ARP3 RNA, where an altered test expression level as  
compared to the control expression level indicates the  
presence of a prostate neoplastic condition in the  
individual. In one embodiment, the sample from the  
individual is prostate tissue. In another embodiment,  
10 the sample from the individual is blood, urine or semen.  
In yet a further embodiment, the ARP3 nucleic acid  
molecule is 15 to 18 nucleotides in length.

In addition, the present invention provides a  
substantially pure ARP3 polypeptide which contains an  
15 amino acid sequence having at least 45% amino acid  
identity with SEQ ID NO:5. In one embodiment, the  
substantially pure ARP3 polypeptide includes the amino  
acid sequence shown as SEQ ID NO:5. The present  
invention also provides a substantially pure ARP3  
20 polypeptide fragment that includes at least eight  
contiguous amino acids of SEQ ID NO:5. Further provided  
by the invention is a binding agent that selectively  
binds an ARP3 polypeptide having at least 45% amino acid  
identity with SEQ ID NO: 5. In one embodiment, the  
25 binding agent that selectively binds an ARP3 polypeptide  
is an antibody.

The present invention also provides a method of  
diagnosing or predicting susceptibility to a prostate  
neoplastic condition in an individual by contacting a  
30 specimen from the individual with a binding agent that  
selectively binds an ARP3 polypeptide having at least 45%

amino acid identity with SEQ ID NO: 5, determining a test expression level of ARP3 polypeptide in the specimen, and comparing the test expression level to a non-neoplastic control expression level of ARP3 polypeptide, where an  
5 altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. A specimen useful in a diagnostic method of the invention can be, for example, prostate tissue, or can be, for example,  
10 blood, serum, urine or serum. A binding agent useful for determining a test expression level of ARP3 polypeptide in a method of the invention can be, for example, an antibody.

The present invention further provides a method  
15 for treating or reducing the severity of a prostate neoplastic condition in an individual by administering to said individual an ARP3 regulatory agent.

Also provided by the invention is a substantially pure ARP4 nucleic acid molecule that  
20 contains a nucleic acid sequence encoding an ARP4 polypeptide having at least 50% amino acid identity with SEQ ID NO:7. In one embodiment, the substantially pure ARP4 nucleic acid molecule contains a nucleic acid sequence encoding the amino acid sequence shown as SEQ ID  
25 NO:7. In another embodiment, the substantially pure ARP4 nucleic acid molecule includes the nucleotide sequence shown as SEQ ID NO:6.

The present invention also provides a substantially pure ARP4 nucleic acid molecule containing  
30 at least 10 contiguous nucleotides of nucleotides 821



to 1940 of SEQ ID NO:6. In one embodiment, the substantially pure ARP4 nucleic acid molecule includes at least 15 contiguous nucleotides of nucleotides 821 to 1940 of SEQ ID NO:6.

5           The present invention further provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from the individual with an ARP4 nucleic acid molecule containing at least 10 contiguous nucleotides of  
10 SEQ ID NO:6, determining a test expression level of ARP4 RNA in the sample, and comparing the test expression level to a non-neoplastic control expression level of ARP4 RNA, where an altered test expression level as compared to the control expression level indicates the  
15 presence of a prostate neoplastic condition in the individual. A sample useful in the invention can be, for example, prostate tissue, or can be, for example, blood, urine or semen. An ARP4 nucleic acid molecule useful in a diagnostic method of the invention can have, for  
20 example, a length of 15 to 18 nucleotides.

          The present invention additionally provides a substantially pure ARP4 polypeptide containing an amino acid sequence having at least 50% amino acid identity with SEQ ID NO:7. Such a substantially pure ARP4  
25 polypeptide can contain, for example, the amino acid sequence shown as SEQ ID NO:7. The present invention also provides a substantially pure ARP4 polypeptide fragment including at least eight contiguous amino acids of SEQ ID NO:7. The invention additionally provides a  
30 binding agent that selectively binds an ARP4 polypeptide having at least 50% amino acid identity with SEQ ID NO:7.

In one embodiment, the binding agent that selectively binds an ARP4 polypeptide is an antibody.

The present invention also provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a specimen from the individual with a binding agent that selectively binds an ARP4 polypeptide having at least 50% amino acid identity with SEQ ID NO: 7, determining a test expression level of ARP4 polypeptide in the specimen, and comparing the test expression level to a non-neoplastic control expression level of ARP4 polypeptide, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. In one embodiment, the specimen from the individual is prostate tissue, and, in another embodiment, the specimen from the individual is blood, serum, urine or semen. A particularly useful binding agent that selectively binds an ARP4 polypeptide is an antibody.

The present invention further provides a method for treating or reducing the severity of a prostate neoplastic condition in an individual by administering to the individual an ARP4 regulatory agent.

Further provided by the invention is a substantially pure ARP5 nucleic acid molecule which contains a nucleic acid sequence encoding an ARP5 polypeptide having at least 40% amino acid identity with SEQ ID NO:9. In one embodiment, the substantially pure ARP5 nucleic acid molecule contains a nucleic acid sequence encoding the amino acid sequence shown as SEQ ID

NO:9. In another embodiment, the substantially pure ARP5 nucleic acid molecule contains the nucleotide sequence shown as SEQ ID NO:8.

The present invention provides a substantially pure ARP5 nucleic acid molecule containing at least 10 contiguous nucleotides of nucleotides 565 to 1276 of SEQ ID NO:8. In one embodiment, the substantially pure ARP5 nucleic acid molecule includes at least 15 contiguous nucleotides of nucleotides 565 to 1276 of SEQ ID NO:8.

In addition, the present invention provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from the individual with an ARP5 nucleic acid molecule which includes at least 10 contiguous nucleotides of SEQ ID NO:8, determining a test expression level of ARP5 RNA in the sample, and comparing the test expression level to a non-neoplastic control expression level of ARP5 RNA, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. In one embodiment, a sample used in a method of the invention is prostate tissue. In another embodiment, a sample used in a method of the invention is blood, urine or semen. In a further embodiment, the ARP5 nucleic acid molecule has a length of 15 to 18 nucleotides.

The present invention also provides a substantially pure ARP5 polypeptide that contains an amino acid sequence having at least 40% amino acid identity with SEQ ID NO:9. In one embodiment, the

substantially pure ARP5 polypeptide contains the amino acid sequence shown as SEQ ID NO:9. The present invention also provides a substantially pure ARP5 polypeptide fragment including at least eight contiguous amino acids of SEQ ID NO:9. Further provided by the invention is a binding agent that selectively binds an ARP5 polypeptide having at least 40% amino acid identity with SEQ ID NO:9, which can be, for example, an antibody.

The present invention also provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a specimen from the individual with a binding agent that selectively binds an ARP5 polypeptide having at least 40% amino acid identity with SEQ ID NO: 9, determining a test expression level of ARP5 polypeptide in the specimen, and comparing the test expression level to a non-neoplastic control expression level of ARP5 polypeptide, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. A specimen useful in the invention can be, for example, prostate tissue, or can be, for example, blood, serum, urine or semen. A binding agent useful in the invention can be, for example, an antibody.

Also provided by the invention is a method for treating or reducing the severity of a prostate neoplastic condition in an individual by administering to the individual an ARP5 regulatory agent.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the nucleotide (SEQ ID NO:1) sequence of ARP1.

Figure 2 shows the nucleotide (SEQ ID NO: 2) and amino acid sequence (SEQ ID NO: 3) of ARP2.

Figure 3 shows the nucleotide (SEQ ID NO:4) and amino acid sequence (SEQ ID NO:5) of ARP3.

Figure 4 shows the nucleotide (SEQ ID NO:6) and amino acid sequence (SEQ ID NO:7) of ARP4.

Figure 5 shows the nucleotide (SEQ ID NO:8) and amino acid (SEQ ID NO:9) sequence of ARP5.

Figure 6 shows multiple tissue Northern blot analysis of ARP4 expression.

**DETAILED DESCRIPTION OF THE INVENTION**

This invention is directed to the discovery of androgen regulated prostate (ARP) expressed nucleic acid molecules. The androgen regulated prostate expressed nucleic acid molecules and encoded gene products are useful as diagnostic markers for neoplastic conditions of the prostate, and, further, are targets for therapy.

As disclosed herein in Example I, the ARP1 cDNA is an androgen-regulated sequence. The ARP1 nucleic acid sequence is disclosed herein in Figure 1. As further disclosed herein, the ARP2 cDNA is another

androgen-regulated cDNA, which contains 4509 nucleotides and is predicted to encode a protein of 252 amino acids (see Figure 2). The androgen-regulated ARP3 cDNA contains 2213 nucleotides and is predicted to encode a protein of 538 amino acids (see Example I and Figure 3). As further disclosed herein, the androgen-regulated ARP4 and ARP5 cDNAs are alternatively spliced mRNA from the same gene. A single nucleotide insertion results in a frameshift change in the coding region. As shown in Figure 4, the ARP4 nucleic acid sequence contains 4433 nucleotides and is predicted to encode a protein of 141 amino acids. The alternatively spliced ARP5 nucleic acid sequence contains 1276 nucleotides and is predicted to encode a protein of 425 amino acids (see Figure 5).

Based on these novel prostate-expressed sequences, the invention provides methods for diagnosing prostate neoplastic conditions. An ARP nucleic acid molecule or polypeptide of the invention can be used alone or in combination with other molecules as a specific marker for prostate cells or prostate neoplastic conditions.

The present invention provides a substantially pure ARP1 nucleic acid molecule containing substantially the nucleotide sequence shown as SEQ ID NO:1. The invention also provides a substantially pure ARP1 nucleic acid molecule containing at least 10 contiguous nucleotides of nucleotides 722 to 1026 of SEQ ID NO:1. In one embodiment, the substantially pure ARP1 nucleic acid molecule includes at least 15 contiguous nucleotides of nucleotides 722 to 1026 of SEQ ID NO:1.

The present invention further provides a substantially pure ARP2 nucleic acid molecule containing substantially the nucleotide sequence shown as SEQ ID NO:2. The invention also provides a substantially pure

5 ARP2 nucleic acid molecule that includes at least 10 contiguous nucleotides of nucleotides 1128 to 4509 of SEQ ID NO:2. In one embodiment, such a substantially pure ARP2 nucleic acid molecule includes at least 15 contiguous nucleotides of nucleotides 1128 to 4509 of SEQ

10 ID NO:2.

The present invention additionally provides a substantially pure ARP3 nucleic acid molecule containing a nucleic acid sequence that encodes an ARP3 polypeptide having at least 45% amino acid identity with SEQ ID NO:5.

15 In one embodiment, the substantially pure ARP3 nucleic acid molecule contains a nucleic acid sequence encoding the amino acid sequence shown as SEQ ID NO:5. In another embodiment, the substantially pure ARP3 nucleic acid molecule contains the nucleotide sequence shown as SEQ ID

20 NO:4.

Also provided by the invention is a substantially pure ARP4 nucleic acid molecule that contains a nucleic acid sequence encoding an ARP4 polypeptide having at least 50% amino acid identity with

25 SEQ ID NO:7. In one embodiment, the substantially pure ARP4 nucleic acid molecule contains a nucleic acid sequence encoding the amino acid sequence shown as SEQ ID NO:7. In another embodiment, the substantially pure ARP4 nucleic acid molecule includes the nucleotide sequence

30 shown as SEQ ID NO:6. The present invention further provides a substantially pure ARP4 nucleic acid molecule

containing at least 10 contiguous nucleotides of  
nucleotides 821 to 1940 of SEQ ID NO:6. Such an ARP4  
nucleic acid molecule can include, for example, at least  
15 contiguous nucleotides of nucleotides 821 to 1940 of  
5 SEQ ID NO:6.

Further provided by the invention is a  
substantially pure ARP5 nucleic acid molecule which  
contains a nucleic acid sequence encoding an ARP5  
polypeptide having at least 40% amino acid identity with  
10 SEQ ID NO:9. In one embodiment, the substantially pure  
ARP5 nucleic acid molecule contains a nucleic acid  
sequence encoding the amino acid sequence shown as SEQ ID  
NO:9. In another embodiment, the substantially pure ARP5  
nucleic acid molecule contains the nucleotide sequence  
15 shown as SEQ ID NO:8. The invention also provides a  
substantially pure ARP5 nucleic acid molecule containing  
at least 10 contiguous nucleotides of nucleotides 565 to  
1276 of SEQ ID NO:8. In one embodiment, the  
substantially pure ARP5 nucleic acid molecule includes at  
20 least 15 contiguous nucleotides of nucleotides 565 to  
1276 of SEQ ID NO:8.

The nucleic acid molecules of the invention  
corresponding to unique sequences are useful in a variety  
of diagnostic procedures which employ probe hybridization  
25 methods. One advantage of employing nucleic acid  
hybridization in diagnostic procedures is that very small  
amounts of sample can be used because the analyte nucleic  
acid molecule can be amplified to many copies by, for  
example, polymerase chain reaction (PCR) or other well  
30 known methods for nucleic acid molecule amplification and  
synthesis.



As used herein, the term "nucleic acid molecule" means a single- or double-stranded DNA or RNA molecule including, for example, genomic DNA, cDNA and mRNA. The term is intended to include nucleic acid molecules of both synthetic and natural origin. A nucleic acid molecule of natural origin can be derived from any animal, such as a human, non-human primate, mouse, rat, rabbit, bovine, porcine, ovine, canine, feline, or amphibian, or from a lower eukaryote. A nucleic acid molecule of the invention can be of linear, circular or branched configuration, and can represent either the sense or antisense strand, or both, of a native nucleic acid molecule. A nucleic acid molecule of the invention can further incorporate a detectable moiety such as a radiolabel, a fluorochrome, a ferromagnetic substance, a luminescent tag or a detectable moiety such as biotin.

As used herein, the term "substantially pure nucleic acid molecule" means a nucleic acid molecule that is substantially free from cellular components or other contaminants that are not the desired molecule. A substantially pure nucleic acid molecule can also be sufficiently homogeneous so as to resolve as a band by gel electrophoresis, and generate a nucleotide sequence profile consistent with a predominant species.

In particular embodiments, the present invention provides a substantially pure ARP1 nucleic acid molecule containing at least 10 contiguous nucleotides of nucleotides 722 to 1026 of SEQ ID NO:1; a substantially pure ARP2 nucleic acid molecule that includes at least 10 contiguous nucleotides of nucleotides 1128 to 4509 of SEQ

Several specific nucleic acid sequences are excluded as nucleic acid molecules of the invention. An ARP1 nucleic acid molecule of the invention containing at least 10 contiguous nucleotides of nucleotides 722 to

Several specific nucleic acid sequences are  
30 excluded as nucleic acid molecules of the invention. An  
ARPl nucleic acid molecule of the invention containing at  
least 10 contiguous nucleotides of nucleotides 722 to

1026 of SEQ ID NO: 1 excludes one or both of AA404252, AI133138, or any subportion thereof. Similarly, an ARP4 nucleic acid molecule of the invention containing at least 10 contiguous nucleotides of nucleotides 821 to 1940 of SEQ ID NO:6 specifically excludes one or any combination of AW861164, AW856874, AI299663, H59488, W60959, AA659693, AW961788, AA249370, AL133779, or any subportion thereof. In addition, an ARP5 nucleic acid molecule of the invention containing at least 10 contiguous nucleotides of nucleotides 565 to 1276 specifically excludes one or any combination of AW861164, AW856874, or BF130410, or a subportion thereof.

In one embodiment, an ARP3 nucleic acid molecule of the invention specifically excludes the nucleotide sequence AK002597. In another embodiment, an ARP4 nucleic acid molecule of the invention specifically excludes the nucleotide sequence AK012931.

A nucleic acid molecule of the invention includes at least 10 contiguous nucleotides corresponding to the reference nucleic acid molecule, and can include at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or at least 25 nucleotides and, if desired, can include at least 30, 40, 50, 100, 300 or 500 nucleotides or up to the full length of the reference nucleic acid molecule. Nucleic acid molecules of such lengths are able to selectively hybridize with the subject nucleic acid molecule in a variety of detection formats described herein.

As used herein, the term "substantially the nucleotide sequence" in reference to a nucleic acid

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mRNA or promoter/regulatory region and be capable of uniquely hybridizing to ARP DNA or RNA.

A nucleic acid molecule of the invention can be produced recombinantly or chemically synthesized using methods well known in the art. Additionally, an ARP nucleic acid molecule can be labeled with a variety of detectable labels including, for example, radioisotopes, fluorescent tags, reporter enzymes, biotin and other ligands for use as a probe in a hybridization method. Such detectable labels can additionally be coupled with, for example, colorimetric or photometric indicator substrate for spectrophotometric detection. Methods for labeling and detecting nucleic acid molecules are well known in the art and can be found described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989), and Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999).

The nucleic acid molecules of the invention can be hybridized under various stringency conditions readily determined by one skilled in the art. Depending on the particular assay, one skilled in the art can readily vary the stringency conditions to optimize detection of an ARP nucleic acid molecule.

In general, the stability of a hybrid is a function of the ion concentration and temperature. Typically, a hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Moderately stringent

hybridization refers to conditions that permit a nucleic acid molecule such as a probe to bind a complementary nucleic acid molecule. The hybridized nucleic acid molecules generally have at least 60% identity, at least  
 5 75% identity, at least 85% identity; or at least 90% identity with the parent or target nucleic acid sequence. Moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhardt's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing  
 10 in 0.2X SSPE, 0.2% SDS, at 42°C. High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

15           The term low stringency hybridization means conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 22°C, followed by washing in 1X SSPE, 0.2% SDS, at 37°C. Denhart's solution contains 1% Ficoll, 1%  
 20 polyvinylpyrrolidine, and 1% bovine serum albumin (BSA). 20X SSPE (sodium chloride, sodium phosphate, ethylene diamide tetraacetic acid (EDTA)) contains 3M sodium chloride, 0.2M sodium phosphate, and 0.025 M (EDTA). Other suitable moderate stringency and high stringency  
 25 hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989); and Ausubel et al., *supra*, 1999). Nucleic  
 30 acid molecules encoding polypeptides hybridize under moderately stringent or high stringency conditions to substantially the entire sequence, or substantial

portions, for example, typically at least 15-30 nucleotides of an ARP nucleic acid sequence.

The invention also provides a modification of an ARP nucleotide sequence that hybridizes to an ARP nucleic acid molecule, for example, an ARP nucleic acid molecule referenced herein as SEQ ID NO:1, 2, 4, 6 or 8, under moderately stringent conditions. Modifications of ARP nucleotide sequences, where the modification has at least 60% identity to an ARP nucleotide sequence, are also provided. The invention also provides modification of an ARP nucleotide sequence having at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, or at least 95% identity to SEQ ID NO: 1, 2, 4, 6 or 8.

Identity of any two nucleic acid sequences can be determined by those skilled in the art based, for example, on a BLAST 2.0 computer alignment, using default parameters. BLAST 2.0 searching is available at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>, as described by Tatiana et al., FEMS Microbiol Lett. 174:247-250 (1999); Altschul et al., Nucleic Acids Res., 25:3389-3402 (1997).

The present invention further provides substantially pure ARP polypeptides encoded by the prostate-expressed nucleic acid molecules of the invention. Thus, the invention provides a substantially pure ARP3 polypeptide which contains an amino acid sequence having at least 45% amino acid identity with SEQ ID NO:5. A substantially pure ARP3 polypeptide of the

5 SEQ ID NO:5.

10 pure ARP4 polypeptide contains the amino acid sequence

20 example, the amino acid sequence shown as SEQ ID NO:9.

30 or ARP5 polypeptide of the invention. It is understood



that, while eight residues is the minimum length of a polypeptide fragment of the invention, a fragment can be longer and can include 9, 10, 11, 12, 13, 14, 15, 18, 20, 25, 30, 35, 40, 45 or more contiguous amino acids of the amino acid sequence shown as SEQ ID NO: 5 in Figure 3, the amino acid sequence shown as SEQ ID NO: 7 in Figure 4, or the amino acid sequence shown a SEQ ID NO: 9 in Figure 5.

The term "ARP3 polypeptide" as used herein, means a polypeptide that is structurally similar to a human ARP3 (SEQ ID NO: 5) and that has at least one biological activity of human ARP3. Such an ARP3 polypeptide has 45% or more amino acid sequence identity to SEQ ID NO:5 and can have, for example, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to human ARP3 (SEQ ID NO:5). Percent amino acid identity can be determined using Clustal W version 1.7 (Thompson et al., Nucleic Acids Res. 22:4673-4680 (1994)).

Thus, it is clear to the skilled person that the term "ARP3 polypeptide" encompasses polypeptides with one or more naturally occurring or non-naturally occurring amino acid substitutions, deletions or insertions as compared to SEQ ID NO:5, provided that the peptide has at least 45% amino acid identity with SEQ ID NO: 5 and retains at least one biological activity of human ARP3. An ARP3 polypeptide can be, for example, a naturally occurring variant of human ARP3 (SEQ ID NO:5); a species homolog including mammalian and non-mammalian homologs and murine, bovine, and primate homologs; an ARP3 polypeptide mutated by recombinant techniques, and

the like. In view of the above definition, it is clear to the skilled person that the *C. elegans* polypeptide encoded by T25F10.5 (T29520), which shares 39.7% amino acid identity with human ARP3 (SEQ ID NO:5), is not encompassed by the invention. In a particular embodiment, the mouse cDNA amino acid sequence encoded by AK002597 is specifically excluded from the definition of an ARP3 polypeptide.

The term "ARP4 polypeptide" as used herein, means a polypeptide that is structurally similar to a human ARP4 (SEQ ID NO: 7) and that has at least one biological activity of human ARP4. Such an ARP4 polypeptide has 50% or more amino acid sequence identity to SEQ ID NO:5 and can have, for example 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more amino acid sequence identity to human ARP4 (SEQ ID NO:7). Percent amino acid identity can be determined using Clustal W version 1.7 as described above.

Thus, the term "ARP4 polypeptide" encompasses polypeptides with one or more naturally occurring or non-naturally occurring amino acid substitutions, deletions or insertions as compared to SEQ ID NO:7, provided that the peptide has at least 50% amino acid identity with SEQ ID NO: 7 and retains at least one biological activity of human ARP4. An ARP4 polypeptide can be, for example, a naturally occurring variant of human ARP4 (SEQ ID NO:7); a species homolog including mammalian and non-mammalian homologs and murine, bovine, and primate homologs; an ARP4 polypeptide mutated by recombinant techniques; and the like. In view of the above definition, it is clear to the skilled person that

5 amino acid sequence encoded by AK012931 is specifically excluded from the definition of an ARP4 polypeptide.

10 biological activity of human ARP5. Such an ARP5 polypeptide has 40% or more amino acid sequence identity to SEQ ID NO:9 and can have, for example 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to human ARP5 (SEQ ID NO:9). Percent amino acid  
15 identity can be determined using Clustal W version 1.7 (Thompson et al., *supra*, 1994).

20 deletions or insertions as compared to SEQ ID NO:9, provided that the peptide has at least 40% amino acid identity with SEQ ID NO: 9 and retains at least one biological activity of human ARP5. An ARP5 polypeptide can be, for example, a naturally occurring variant of

25 human ARP5 (SEQ ID NO:9); a species homolog including mammalian and non-mammalian homologs and murine, bovine, and primate homologs; an ARP4 polypeptide mutated by recombinant techniques, and the like. In view of the above definition, it is clear to the skilled person that

30 the *Drosophila* polypeptide encoded by AE003831 (AAF58858), which shares 35% amino acid identity with

human ARP5 (SEQ ID NO:9), is not encompassed by the invention.

Modifications to ARP3, ARP4 and ARP5 polypeptides of SEQ ID NOS:5, 7, or 9 that are  
5 encompassed within the invention include, for example, an addition, deletion, or substitution of one or more conservative or non-conservative amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties  
10 such as amino or acetyl groups.

The present invention provides a binding agent that selectively binds an ARP3 polypeptide having at least 45% amino acid identity with SEQ ID NO:5. The present invention also provides a binding agent that  
15 selectively binds an ARP4 polypeptide having at least 50% amino acid identity with SEQ ID NO:7. Further provided by the invention is a binding agent that selectively binds an ARP5 polypeptide having at least 40% amino acid identity with SEQ ID NO:9. Particularly useful binding  
20 agents of the invention are polyclonal and monoclonal antibodies and binding portions thereof.

As used herein, the term "binding agent" when used in reference to a specified ARP polypeptide, means a compound, including a simple or complex organic molecule,  
25 a metal containing compound, carbohydrate, peptide, protein, peptidomimetic, glycoprotein, lipoprotein, lipid, nucleic acid molecule, antibody, or the like that selectively binds the specified ARP3, ARP4 or ARP5 polypeptide, or fragment thereof. For example, a binding  
30 agent can be a polypeptide that selectively binds with

high affinity or avidity to the specified ARP polypeptide, without substantial cross-reactivity to other unrelated polypeptides. The affinity of a binding agent that selectively binds an ARP polypeptide generally is greater than about  $10^5 \text{ M}^{-1}$  and can be greater than about  $10^6 \text{ M}^{-1}$ . A binding agent also can bind with high affinity; such an agent generally binds with an affinity greater than  $10^8 \text{ M}^{-1}$  to  $10^9 \text{ M}^{-1}$ . Specific examples of such selective binding agents include a polyclonal or monoclonal antibody selective for an ARP3, ARP4 or ARP5 polypeptide, or a fragment thereof containing at least eight contiguous amino acids of SEQ ID NO: 5, 7 or 9; or a nucleic acid molecule, nucleic acid analog, or small organic molecule, identified, for example, by affinity screening of the appropriate library. For certain applications, a binding agent can be utilized that preferentially recognizes a particular conformational or post-translationally modified state of the specified ARP polypeptide. The binding agent can be labeled with a detectable moiety, if desired, or rendered detectable by specific binding to a detectable secondary binding agent.

As used herein, the term "antibody" is used in its broadest sense to mean polyclonal and monoclonal antibodies, including antigen binding fragments of such antibodies. As used herein, the term antigen means a native or synthesized fragment of a polypeptide of the invention. Such an antibody of the invention, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for the specified ARP3, ARP4 or ARP5 polypeptide, or fragment thereof, of at least about  $1 \times 10^5 \text{ M}^{-1}$ . Thus, Fab,  $\text{F(ab')}_2$ , Fd and Fv fragments of an anti-ARP antibody, which retain specific

binding activity for an ARP polypeptide of the invention, or fragment thereof, are included within the definition of an antibody. Specific binding activity can be readily determined by one skilled in the art, for example, by

5 comparing the binding activity of the antibody to the specified ARP polypeptide, or fragment thereof, versus a control polypeptide that does not include a polypeptide of the invention. Methods of preparing polyclonal or monoclonal antibodies are well known to those skilled in

10 the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

The term antibody also includes naturally occurring antibodies as well as non-naturally occurring

15 antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced

20 recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al. (Science 246:1275-1281 (1989)). These and other methods of making, for example, chimeric, humanized,

25 CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989) ; Harlow and Lane, *supra*, 1988); Hilyard et al., Protein Engineering: A practical

30 approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995)).

Methods of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual further are provided by the invention. In particular, the present invention provides a method of  
30 diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from the individual with an ARPl nucleic acid

Methods of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual further are provided by the invention. In particular, the present invention provides a method of  
30 diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from the individual with an ARPl nucleic acid

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ARP3 RNA, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. An ARP3 nucleic acid molecule useful in the invention can contain, for example 15 to 18 nucleotides.

The present invention further provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from said individual with an ARP4 nucleic acid molecule containing at least 10 contiguous nucleotides of SEQ ID NO:6, determining a test expression level of ARP4 RNA in the sample, and comparing the test expression level to a non-neoplastic control expression level of ARP4 RNA, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. In one embodiment, the ARP4 nucleic acid molecule is 15 to 18 nucleotides in length.

Further provided by the invention is a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a sample from said individual with an ARP5 nucleic acid molecule includes at least 10 contiguous nucleotides of SEQ ID NO:8, determining a test expression level of ARP5 RNA in the sample, and comparing the test expression level to a non-neoplastic control expression level of ARP5 RNA, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual. In one embodiment the ARP5 nucleic acid molecule is 15 to 18 nucleotides in length.

5 determined, for example, using a normal prostate cell or  
an androgen-dependent cell line.

As described herein, the term "prostate neoplastic condition" means a benign or malignant or metastatic prostate lesion of proliferating cells. For example, primary prostate tumors are classified into stages TX, T0, T1, T2, T3, and T4. Metastatic prostate cancer is classified into stages D1, D2, and D3. The term further includes prostate neoplasm. Each of the above conditions is encompassed within the term "prostate neoplastic condition."

As used herein, the term "sample" means any biological fluid, cell, tissue, organ or portion thereof, that includes or potentially includes an ARP nucleic acid molecule. The term sample includes materials present in an individual as well as materials obtained or derived from the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid molecule. A sample can be prepared by methods known in the art suitable for the particular format of the detection method.

As used herein, the term "test expression  
30 level" is used in reference to ARP RNA expression or to

ARP polypeptide expression as discussed below and means the extent, amount or rate of synthesis of the specified ARP RNA or polypeptide. The amount or rate of synthesis can be determined by measuring the accumulation or  
5 synthesis of the specified ARP RNA or polypeptide, or by measuring an activity associated with a polypeptide of the invention.

As used herein, an "altered test expression level" means a test expression level that is either  
10 elevated or reduced as compared to a control expression level. One skilled in the art understands that such an elevation or reduction is not within the inherent variability of the assay and generally is an expression level that is at least two-fold elevated or reduced. An  
15 altered test expression level can be, for example, two-fold, five-fold, ten-fold, 100-fold, 200-fold, or 1000-fold increased in the extent, amount or rate of synthesis of the specified RNA or polypeptide as compared to a control expression level of the specified ARP RNA or  
20 polypeptide. An altered test expression level also can be, for example, two-fold, five-fold, ten-fold, 100-fold, 200-fold, or 1000-fold decreased in the extent, amount or rate of synthesis of the specified ARP RNA or polypeptide compared to a control expression level of the same ARP  
25 RNA or polypeptide.

As used herein, the term "non-neoplastic control expression level" means an ARP RNA expression level or to an ARP polypeptide expression level as discussed below used as a baseline for comparison to a  
30 test expression level. For example, a suitable control expression level can be the expression level of ARP

nucleic acid or polypeptide from a non-neoplastic prostate cell or a fluid sample obtained from a normal individual. Another suitable non-neoplastic control is a prostate cell line that is androgen-dependent. It is

5 understood that ARP nucleic acid or polypeptide expression levels determined in cell lines generally are determined under androgen-depleted growth conditions which can correlate to non-neoplastic control expression levels. The response of an androgen-depleted androgen-  
10 dependent prostate cell line to androgen stimulation will be indicative of ARP nucleic acid or polypeptide expression levels in neoplastic cells. The control expression level can be determined simultaneously with one or more test samples or, alternatively, expression  
15 levels can be established for a particular type of sample and standardized to internal or external parameters such as protein or nucleic acid content, cell number or mass of tissue. Such standardized control samples can then be directly compared with results obtained from the test  
20 sample. As indicated above, an increase of two-fold or more, for example, of a test expression level of the specified ARP nucleic acid or polypeptide indicates the presence of a prostate neoplastic condition or pathology in the tested individual.

25 A detectable label can be useful in a method of the invention and refers to a molecule that renders a nucleic acid molecule of the invention detectable by an analytical method. An appropriate detectable label depends on the particular assay format; such labels are  
30 well known by those skilled in the art. For example, a detectable label selective for a nucleic acid molecule can be a complementary nucleic acid molecule, such as a

hybridization probe, that selectively hybridizes to the nucleic acid molecule. A hybridization probe can be labeled with a measurable moiety, such as a radioisotope, fluorochrome, chemiluminescent marker, biotin, or other moiety known in the art that is measurable by analytical methods. A detectable label also can be a nucleic acid molecule without a measurable moiety. For example, PCR or RT-PCR primers can be used without conjugation to selectively amplify all or a desired portion of the nucleic acid molecule. The amplified nucleic acid molecules can then be detected by methods known in the art.

The present invention also provide diagnostic methods that rely on a binding agent that selectively binds the specified ARP. A method of the invention for diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual is practiced by contacting a specimen from an individual with a binding agent that selectively binds an ARP3 polypeptide having at least 45% amino acid identity with SEQ ID NO:5; determining a test expression level of ARP3 polypeptide in the specimen; and comparing the test expression level to a non-neoplastic control expression level of ARP3 polypeptide, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual.

The present invention also provides a method for diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a specimen from the individual with a binding agent that

selectively binds an ARP4 polypeptide having at least 50% amino acid identity with SEQ ID NO:7; determining a test expression level of ARP4 polypeptide in the specimen; and comparing the test expression level to a non-neoplastic control expression level of ARP4 polypeptide, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual.

Further provided by the invention is a method for diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by contacting a specimen from an individual with a binding agent that selectively binds an ARP5 polypeptide having at least 40% amino acid identity with SEQ ID NO:9; determining a test expression level of ARP5 polypeptide in the specimen; and comparing the test expression level to a non-neoplastic control expression level of ARP5 polypeptide, where an altered test expression level as compared to the control expression level indicates the presence of a prostate neoplastic condition in the individual.

In a method of the invention, the specimen can contain, for example, a prostate cell or prostate tissue and, in one embodiment, is a fluid such as blood, serum, urine or semen. The control expression level can be determined, for example, using a normal prostate cell or an androgen-dependent cell line. In addition, a binding agent selective for a polypeptide of the invention can be, for example, an antibody, and, if desired, can further include a detectable label.

5 includes materials present in an individual as well as materials obtained or derived from the individual. For example, a specimen can be a histologic section obtained by biopsy, or cells that are placed in or adapted to tissue culture. A specimen further can be a subcellular  
10 fraction or extract, or a crude or substantially pure protein preparation. A specimen can be prepared by methods known in the art suitable for the particular format of the detection method.

In methods of the invention, the specimen can be, for example, a prostate cell or prostate tissue such as a tissue biopsy. A specimen can also be a fluid sample, for example, blood, serum, urine or semen. A normal specimen can be, for example, a normal prostate cell or an androgen-dependent cell line.

20           These diagnostic methods of the invention rely  
on a binding agent. As described above, the term  
"binding agent" when used in reference to an ARP  
polypeptide, is intended to mean a compound, including a  
simple or complex organic molecule, a metal containing  
25 compound, carbohydrate, peptide, protein, peptidomimetic,  
glycoprotein, lipoprotein, lipid, nucleic acid molecule,  
antibody, or the like that selectively binds the  
specified ARP polypeptide, or fragment thereof. The  
binding agent can be labeled with a detectable moiety, if  
30 desired, or rendered detectable by specific binding to a

detectable secondary binding agent. Exemplary binding agents are discussed hereinabove.

A prostate neoplastic condition is a benign or malignant prostate lesion of proliferating cells. Prostate neoplastic conditions include, for example, prostate interepithelial neoplasia (PIN) and prostate cancer. Prostate cancer is an uncontrolled proliferation of prostate cells which can invade and destroy adjacent tissues as well as metastasize. Primary prostate tumors can be classified into stages TX, T0, T1, T2, T3, and T4 and metastatic tumors can be classified into stages D1, D2 and D3. Similarly, there are classifications known by those skilled in the art for the progressive stages of precancerous lesions or PIN. The methods herein are applicable for the diagnosis or treatment of any or all stages of prostate neoplastic conditions.

The methods of the invention are also applicable to prostate pathologies other than neoplastic conditions. Such other pathologies include, for example, benign prostatic hyperplasia (BPH) and prostatitis. BPH is one of the most common diseases in adult males. Histological evidence of BPH has been found in more than 40% of men in their fifties and almost 90% of men in their eighties. The disease results from the accumulation of non-malignant nodules arising in a small region around the proximal segment of the prostatic urethra which leads to an increase in prostate volume. If left untreated, BPH can result in acute and chronic retention of urine, renal failure secondary to obstructive uropathy, serious urinary tract infection and irreversible bladder decompensation. Prostatitis is an



infection of the prostate. Other prostate pathologies known to those skilled in the art exist as well and are similarly applicable for diagnosis or treatment using the methods of the invention. Various neoplastic conditions of the prostate as well as prostate pathologies can be found described in, for example, Campbell's Urology, Seventh Edition, W.B. Saunders Company, Philadelphia (1998). Therefore, the methods of the invention are applicable to both prostate neoplastic conditions and prostate pathologies.

Therefore, the invention provides a method for both diagnosing and prognosing a prostate neoplastic condition including prostate cancer and prostate interepithelial neoplasia as well as other prostate pathologies such as BPH and prostatitis.

The invention provides a method of diagnosing or predicting prostate neoplastic conditions based on a finding of a positive correlation between a test expression level of an ARP polypeptide or nucleic acid in neoplastic cells of the prostate and the degree or extent of the neoplastic condition or pathology. The diagnostic methods of the invention are applicable to numerous prostate neoplastic conditions and pathologies as described above. One consequence of progression into these neoplastic and pathological conditions can be altered expression of ARP polypeptide or nucleic acid in prostate tissue. The alteration in ARP polypeptide or nucleic acid expression in individuals suffering from a prostate neoplastic condition can be measured by comparing the amount of ARP polypeptide or nucleic acid to that found, for example, in normal prostate tissue

samples or in normal blood or serum samples. A two-fold or more increase or decrease in a test expression level in a prostate cell sample relative to a non-neoplastic control expression sample obtained, for example, from  
5 normal prostate cells or from an androgen-dependent cell line is indicative of a prostate neoplastic condition or pathology. Similarly, an alteration in ARP polypeptide or nucleic acid expression leading to an increased or decreased secretion into the blood or other circulatory  
10 fluids of the individual compared to a non-neoplastic control blood or fluid samples also can be indicative of a prostate neoplastic condition or pathology. For example, an alteration in ARP polypeptide or nucleic acid expression can lead to a two-fold, five-fold, ten-fold,  
15 100-fold, 200-fold or 1000-fold increased secretion into the blood or other circulatory fluids of the individual compared to a non-neoplastic control blood or fluid samples. As another example, an alteration in ARP polypeptide or nucleic acid expression can lead to a  
20 two-fold, five-fold, ten-fold, 100-fold, 200-fold or 1000-fold decreased secretion into the blood or other circulatory fluids of the individual compared to a non-neoplastic control blood or fluid samples.

As a diagnostic indicator, an ARP polypeptide  
25 or nucleic acid molecule can be used qualitatively to positively identify a prostate neoplastic condition or pathology as described above. Alternatively, ARP polypeptide or nucleic acid molecule also can be used quantitatively to determine the degree or susceptibility  
30 of a prostate neoplastic condition or pathology. For example, successive increases or decreases in the expression levels of ARP polypeptide or nucleic acid can

be used as a predictive indicator of the degree or severity of a prostate neoplastic condition or pathology. For example, increased expression can lead to a rise in accumulated levels and can be positively correlated with

5 increased severity of a neoplastic condition of the prostate. A higher level of ARP polypeptide or nucleic acid expression can be correlated with a later stage of a prostate neoplastic condition or pathology. For example, increases in expression levels of two-fold or more

10 compared to a normal sample can be indicative of at least prostate neoplasia. ARP polypeptide or nucleic acid molecule also can be used quantitatively to distinguish between pathologies and neoplastic conditions as well as to distinguish between the different types of neoplastic

15 conditions.

Correlative alterations can be determined by comparison of ARP polypeptide or nucleic acid expression from the individual having, or suspected of having, a neoplastic condition of the prostate to expression levels

20 of ARP polypeptide or nucleic acid from known specimens or samples determined to exhibit a prostate neoplastic condition. Alternatively, correlative alterations also can be determined by comparison of a test expression level of ARP polypeptide or nucleic acid expression to

25 expression levels of other known markers of prostate cancer such as prostate specific antigen (PSA), glandular kallikrein 2 (hK2) and prostase/PRSS18. These other known markers can be used, for example, as an internal or external standard for correlation of stage-specific

30 expression with altered ARP polypeptide or nucleic acid expression and severity of the neoplastic or pathological condition. Conversely, a regression in the severity of a

prostate neoplastic condition or pathology can be followed by a corresponding reversal in ARP polypeptide or nucleic acid expression levels and can similarly be assessed using the methods described herein.

5           Given the teachings and guidance provided  
herein, those skilled in the art will know or can  
determine the stage or severity of a prostate neoplastic  
condition or pathology based on a determination of ARP  
polypeptide or nucleic acid expression and correlation  
10 with a prostate neoplastic condition or pathology. A  
correlation can be determined using known procedures and  
marker comparisons as described herein. For a review of  
recognized values for such other marker in normal versus  
pathological tissues, see, for example, Campbell's  
15 Urology, Seventh Edition, W.B. Saunders Company,  
Philadelphia (1998).

The use of ARP polypeptide or nucleic acid expression levels in prostate cells, the circulatory system and urine as a diagnostic indicator of a prostate pathology allows for early diagnosis as a predictive indicator when no physiological or pathological symptoms are apparent. The methods are particularly applicable to any males over age 50, African-American males and males with familial history of prostate neoplastic conditions or pathologies. The diagnostic methods of the invention also are particularly applicable to individuals predicted to be at risk for prostate neoplastic conditions or pathologies by reliable prognostic indicators prior to onset of overt clinical symptoms. All that is necessary is to determine the ARP polypeptide or nucleic acid prostate tissue or circulatory or bodily fluid expression

levels to determine whether there is altered ARP polypeptide or nucleic acid levels in the individual suspected of having a prostate pathology compared to a control expression level such as the level observed in  
5 normal individuals. Those skilled in the art will know by using routine examinations and practices in the field of medicine those individuals who are applicable candidates for diagnosis by the methods of the invention.

For example, individuals suspected of having a  
10 prostate neoplastic condition or pathology can be identified by exhibiting presenting signs of prostate cancer which include, for example, a palpable nodule (> 50% of the cases), dysuria, cystitis and prostatitis, frequency, urinary retention, or decreased urine stream.  
15 Signs of advanced disease include pain, uremia, weight loss and systemic bleeding. Prognostic methods of this invention are applicable to individuals after diagnosis of a prostate neoplastic condition, for example, to monitor improvements or identify residual neoplastic  
20 prostate cells using, for example, imaging methods known in the art and which target ARP polypeptide or nucleic acid. Therefore, the invention also provides a method of predicting the onset of a prostate neoplastic condition or pathology by determining an altered test expression  
25 level of one of the ARP nucleic acid molecules or polypeptides of the invention.

The diagnostic methods of the invention are applicable for use with a variety of different types of samples or specimens isolated or obtained from an  
30 individual having, or suspected of having a prostate neoplastic condition or prostate pathology. For example,

A tissue or cell sample or specimen can be obtained, for example, by biopsy or surgery. As described below, and

Additionally, when combined with amplification methods

amplification of the signal with enzymatic coupling or photometric enhancement can be employed using only a few or a small number of cells.

Whole tissue obtained from a prostate biopsy or surgery is one example of a prostate cell sample or specimen. Whole tissue prostate cell samples or specimens can be assayed employing any of the formats described below. For example, the prostate tissue sample can be mounted and hybridized *in situ* with ARP nucleic acid probes. Similar histological formats employing protein detection methods and *in situ* activity assays also can be used to detect an ARP polypeptide in whole tissue prostate cell specimens. Protein detection methods include, for example, staining with an ARP specific antibody and activity assays. Such histological methods as well as others well known to those skilled in the art are applicable for use in the diagnostic methods of the invention using whole tissue as the source of a

prostate cell specimen. Methods for preparing and mounting the samples and specimens are similarly well known in the art.

Individual prostate cells and cell aggregates from an individual having, or suspected of having a prostate neoplastic condition or pathology also are prostate cell samples which can be analyzed for an altered test expression level in a method of the invention. The cells can be grown in culture and analyzed *in situ* using procedures such as those described above. Whole cell samples expressing cell surface markers associated with ARP polypeptide or nucleic acid expression can be rapidly tested using fluorescent or magnetic activated cell sorting (FACS or MACS) with labeled binding agents selective for the surface marker or using binding agents selective for epithelial or prostate cell populations, for example, and then determining a test expression level of a specified ARP polypeptide or nucleic acid within this population. The test expression level can be determined using, for example, binding agents selective for polypeptides of the invention or by hybridization to a specific nucleic acid molecule of the invention. Other methods for measuring the expression level of ARP polypeptide or nucleic acid in whole cell samples are known in the art and are similarly applicable in any of the diagnostic formats described below.

The tissue or whole cell prostate cell sample or specimen obtained from an individual also can be analyzed for increased ARP polypeptide or nucleic acid expression by lysing the cell and measuring a test

expression levels of ARP polypeptide or nucleic acid in the lysate, a fractionated portion thereof or a purified component thereof using any of diagnostic formats described herein. For example, if a hybridization format is used, ARP RNA can be amplified directly from the lysate using PCR, or other amplification procedures well known in the art such as RT-PCR, 5' or 3' RACE to directly measure the expression levels of ARP nucleic acid molecules. RNA also can be isolated and probed directly such as by solution hybridization or indirectly by hybridization to immobilized RNA. Similarly, when determining a test expression level of ARP using polypeptide detection formats, lysates can be assayed directly, or they can be further fractionated to enrich for ARP polypeptide and its corresponding activity. Numerous other methods applicable for use with whole prostate cell samples are well known to those skilled in the art and can accordingly be used in the methods of the invention.

20           The prostate tissue or cell sample or specimen can be obtained directly from the individual or, alternatively, it can be obtained from other sources for testing. Similarly, a cell sample can be tested when it is freshly isolated or it can be tested following short or prolonged periods of cryopreservation without substantial loss in accuracy or sensitivity. If the sample is to be tested following an indeterminate period of time, it can be obtained and then cryopreserved, or stored at 4°C for short periods of time, for example. An advantage of the diagnostic methods of the invention is that they do not require histological analysis of the sample. As such, the sample can be initially



disaggregated, lysed, fractionated or purified and the active component stored for later diagnosis.

The diagnostic methods of the invention are applicable for use with a variety of different types of samples and specimens other than prostate cell samples. For example, an ARP polypeptide or fragment thereof that is released into the extracellular space, including circulatory fluids as well as other bodily fluids, can be detected in a method of the invention. In such a case, the diagnostic methods of the invention are practiced with fluid samples collected from an individual having, or suspected of having a neoplastic condition of the prostate or a prostate pathology.

Fluid samples and specimens, which can be measured for ARP polypeptide or nucleic acid expression levels, include, for example, blood, serum, lymph, urine and semen. Other bodily fluids are known to those skilled in the art and are similarly applicable for use as a sample or specimen in the diagnostic methods of the invention. One advantage of analyzing fluid samples or specimens is that they are readily obtainable, in sufficient quantity, without invasive procedures as required by biopsy and surgery. Analysis of fluid samples or specimens such as blood, serum and urine will generally be in the diagnostic formats described herein which measure ARP polypeptide levels or activity. As the ARP related polypeptide is circulating in a soluble form, the methods will be similar to those which measure expression levels from cell lysates, fractionated portions thereof or purified components.

Prostate neoplastic conditions and prostate pathologies can be diagnosed, predicted or prognosed by measuring a test expression level of ARP polypeptide or nucleic acid in a prostate cell sample, circulating fluid or other bodily fluid obtained from the individual. As described herein, a test or control expression level can be measured by a variety of methods known in the art. For example, a test expression level of a specified ARP can be determined by measuring the amount of ARP RNA or polypeptide in a sample or specimen from the individual. Alternatively, a test expression level of ARP can be determined by measuring the amount of an ARP activity in a specimen, the amount of activity being indicative of the specified ARP polypeptide expression level.

One skilled in the art can readily determine an appropriate assay system given the teachings and guidance provided herein and choose a method based on measuring ARP RNA, polypeptide or activity. Considerations such as the sample or specimen type, availability and amount will also influence selection of a particular diagnostic format. For example, if the sample or specimen is a prostate cell sample and there is only a small amount available, then diagnostic formats which measure the amount of ARP RNA by, for example, PCR amplification, or which measure ARP-related cell surface polypeptide by, for example, FACS analysis can be appropriate choices for determining a test expression level. Alternatively, if the specimen is a blood sample and the user is analysing numerous different samples simultaneous, such as in a clinical setting, then a multisample format, such as an Enzyme Linked Immunoabsorbant Assay (ELISA), which measures the amount of an ARP polypeptide can be an

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5 Multisample formats such as an ELISA or two-dimensional  
array offer the advantage of analyzing numerous,  
different samples in a single assay. Solid-phase dip  
stick-based methods offer the advantage of being able to  
rapidly analyze a patient's fluid sample and obtain an  
10 immediate result.

Nucleic acid molecules useful for measuring a test expression level of a specified ARP RNA are disclosed herein above. Briefly, for detection by hybridization, an ARP nucleic acid molecule having a detectable label is added to a prostate cell sample or a fluid sample obtained from the individual having, or suspected of having a prostate neoplastic condition or pathology under conditions which allow annealing of the molecule to an ARP RNA. Methods for detecting ARP RNA in a sample can include the use of, for example, RT-PCR. Conditions are well known in the art for both solution and solid phase hybridization procedures. Moreover, optimization of hybridization conditions can be performed, if desired, by hybridization of an aliquot of the sample at different temperatures, durations and in different buffer conditions. Such procedures are routine and well known to those skilled in the art. Following annealing, the sample is washed and the signal is measured and compared with a suitable control or standard value. The magnitude of the hybridization signal is directly proportional to the expression levels of ARP RNA.

The diagnostic procedures described herein can additionally be used in conjunction with other prostate markers, such as prostate specific antigen, human glandular kallikrein 2 (hk2) and prostase/PRSS18 for simultaneous or independent corroboration of a sample. Additionally, ARP polypeptide or nucleic acid expression can be used, for example, in combination with other markers to further distinguish normal basal cells, secretory cells and neoplastic cells of the prostate. Moreover, ARP polypeptide or nucleic acid expression can be used in conjunction with smooth muscle cell markers to distinguish between pathological conditions such as benign prostate hypertrophy (BPH) and neoplasia. Those skilled in the art will know which markers are applicable for use in conjunction with ARP polypeptide or nucleic acid to delineate more specific diagnostic information such as that described above.

The invention also provides diagnostic methods based on determining whether there is an altered test expression level of an ARP3, ARP4 or ARP5 polypeptide using a binding agent that selectively binds the recited polypeptide. Essentially all modes of affinity binding assays are applicable for use in determining a test expression level of an ARP polypeptide in a method of the invention. Such methods are rapid, efficient and sensitive. Moreover, affinity binding methods are simple and can be modified to be performed under a variety of clinical settings and conditions to suit a variety of particular needs. Affinity binding assays which are known and can be used in the methods of the invention include both soluble and solid phase formats. A specific example of a soluble phase affinity binding assay is

immunoprecipitation using an ARP selective antibody or other binding agent. Solid phase formats are advantageous in that they are rapid and can be performed easily and simultaneously on multiple different samples without losing sensitivity or accuracy. Moreover, solid phase affinity binding assays are further amenable to high throughput and ultra high throughput screening and automation.

Specific examples of solid phase affinity binding assays include immunoaffinity binding assays such as an ELISA and radioimmune assay (RIA). Other solid phase affinity binding assays are known to those skilled in the art and are applicable to the methods of the invention. Although affinity binding assays are generally formatted for use with an antibody binding molecule that is selective for the analyte or ligand of interest, essentially any binding agent can be alternatively substituted for the selectively binding antibody. Such binding agents include, for example, macromolecules such as polypeptides, peptides, nucleic acid molecules, lipids and sugars as well as small molecule compounds. Methods are known in the art for identifying such molecules which bind selectively to a particular analyte or ligand and include, for example, surface display libraries and combinatorial libraries. Thus, for a molecule other than an antibody to be used in an affinity binding assay, all that is necessary is for the binding agent to exhibit selective binding activity for a polypeptide of the invention.

Various modes of affinity binding formats are similarly known which can be used in the diagnostic

methods of the invention. For the purpose of illustration, particular embodiments of such affinity binding assays will be described further in reference to immunoaffinity binding assays. The various modes of affinity binding assays, such as immunoaffinity binding assays, include, for example, solid phase ELISA and RIA as well as modifications thereof. Such modifications thereof include, for example, capture assays and sandwich assays as well as the use of either mode in combination with a competition assay format. The choice of which mode or format of immunoaffinity binding assay to use will depend on the intent of the user. Such methods can be found described in common laboratory manuals such as Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1999).

As with the hybridization methods described previously, the diagnostic formats employing affinity binding can be used in conjunction with a variety of detection labels and systems known in the art to quantitate amounts of a polypeptide of the invention in the analyzed sample. Detection systems include the detection of bound polypeptide on the invention by both direct and indirect means. Direct detection methods include labeling of the ARP-selective antibody or binding agent. Indirect detection systems include, for example, the use of labeled secondary antibodies and binding agents.

Secondary antibodies, labels and detection systems are well known in the art and can be obtained commercially or by techniques well known in the art. The detectable labels and systems employed with the

ARP-selective binding agent should not impair binding of the agent to the corresponding ARP polypeptide.

Moreover, multiple antibody and label systems can be employed for detecting the bound ARP-selective antibody

- 5 to enhance the sensitivity of the binding assay if desired.

As with the hybridization formats described previously, detectable labels can be essentially any label that can be quantitated or measured by analytical  
10 methods. Such labels include, for example, enzymes, radioisotopes, fluorochromes as well as chemi- and bioluminescent compounds. Specific examples of enzyme labels include horseradish peroxidase (HRP), alkaline phosphatase (AP),  $\beta$ -galactosidase, urease and luciferase.

- 15 A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable by measuring absorbance at 450 nm. An  
20 alkaline phosphatase detection system can be used with the chromogenic substrate *p*-nitrophenyl phosphate, for example, which yields a soluble product readily detectable by measuring absorbance at 405 nm. Similarly, a  $\beta$ -galactosidase detection system can be used with the  
25 chromogenic substrate *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG), which yields a soluble product detectable by measuring absorbance at 410 nm, or a urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals, St.  
30 Louis, MO). Luciferin is the substrate compound for



Fluorochrome detection labels are rendered detectable through the emission of light of ultraviolet or visible wavelength after excitation by light or another energy source. DAPI, fluorescein, Hoechst 33258, R-phycoerythrin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red and lissamine are specific examples of fluorochrome detection labels that can be utilized in the affinity binding formats of the invention. A particularly useful fluorochrome is fluorescein or rhodamine.

Alternatively, radioisotopes can be used as detectable labels in the methods of the invention. Iodine-125 is a specific example of a radioisotope useful as a detectable label.

Signals from detectable labels can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a fluorometer to detect fluorescence in the presence of light of a certain wavelength; or a radiation counter to detect radiation, such as a gamma counter for detection of iodine-125. For detection of an enzyme-linked secondary antibody, for example, a quantitative analysis of the amount of bound

agent can be made using a spectrophotometer such as an  
EMAX Microplate Reader (Molecular Devices, Menlo Park,  
CA) in accordance with the manufacturer's instructions.  
If desired, the assays of the invention can be automated  
5 or performed robotically, and the signal from multiple  
samples can be detected simultaneously.

The diagnostic formats of the present invention  
can be forward, reverse or simultaneous as described in  
U.S. Patent No. 4,376,110 and No. 4,778,751. Separation  
10 steps for the various assay formats described herein,  
including the removal of unbound secondary antibody, can  
be performed by methods known in the art (Harlow and  
Lane, *supra*). For example, washing with a suitable  
buffer can be followed by filtration, aspiration, vacuum  
15 or magnetic separation as well as by centrifugation.

A binding agent selective for an ARP  
polypeptide also can be utilized in imaging methods that  
are targeted at ARP expressing prostate cells. These  
imaging techniques have utility in identification of  
20 residual neoplastic cells at the primary site following  
standard treatments including, for example, radical  
prostatectomy, radiation or hormone therapy. In  
addition, imaging techniques that detect neoplastic  
prostate cells have utility in detecting secondary sites  
25 of metastasis. A binding agent that selectively binds  
ARP3, ARP4 or ARP5 can be radiolabeled with, for example,  
<sup>111</sup>indium and infused intravenously as described by Kahn  
et al., Journal of Urology 152:1952-1955 (1994). The  
binding agent selective for an ARP polypeptide can be,  
30 for example, a monoclonal antibody selective for an ARP  
polypeptide. Imaging can be accomplished by, for

example, radioimmunoscentigraphy as described by Kahn et al., *supra*.

In one embodiment, the invention provides a method of diagnosing or predicting the susceptibility of a prostate neoplastic condition in an individual suspected of having a neoplastic condition of the prostate, where a test expression level of an ARP polypeptide is determined by measuring the amount of ARP3, ARP4, or ARP5 polypeptide activity. The method is practiced by contacting a specimen from the individual with an agent that functions to measure an activity associated with an ARP3, ARP4, or ARP5 polypeptide of the invention.

As with the hybridization and affinity binding formats described above, activity assays similarly can be performed using essentially identical methods and modes of analysis. Therefore, solution and solid phase modes, including multisample ELISA, RIA and two-dimensional array procedures are applicable for use in measuring an activity associated with an ARP polypeptide. The activity can be measured by, for example, incubating an agent that functions to measure an activity associated with an ARP polypeptide with the sample and determining the amount of product formed that corresponds to ARP3, ARP4 or ARP5 polypeptide activity. The amount of product formed will directly correlate with the ARP3, ARP4 or ARP5 polypeptide activity in the specimen and therefore, with the expression levels of the corresponding polypeptide of the invention in the specimen.

1 The invention further provides a method of  
identifying a compound that inhibits ARP3, ARP4 or ARP5  
polypeptide activity. The method consists of contacting  
a specimen containing an ARP polypeptide and an agent  
5 that functions to measure an activity associated with an  
ARP polypeptide with a test compound under conditions  
that allow formation of a product that corresponds to an  
ARP polypeptide activity and measuring the amount of  
product formed, where a decrease in the amount of product  
10 formed in the presence of the test compound compared to  
the absence of the test compound indicates that the  
compound has ARP polypeptide inhibitory activity.  
Similarly, compounds that increase the activity of an ARP  
polypeptide also can be identified. A test compound  
15 added to a specimen containing an ARP polypeptide and an  
agent that functions to measure an activity associated  
with an ARP polypeptide which increases the amount of  
product formed compared to the absence of the test  
compound indicates that the compound increases the  
20 corresponding ARP polypeptide activity. Therefore, the  
invention provides a method of identifying compounds that  
modulate the activity of an ARP polypeptide. The ARP  
polypeptide containing specimen used for such a method  
can be serum, prostate tissue, a prostate cell population  
25 or a recombinant cell population expressing an ARP  
polypeptide.

Those compounds having inhibitory activity are  
considered as potential ARP polypeptide antagonists and  
further as potential therapeutic agents for treatment of  
30 neoplastic conditions of the prostate. Similarly, those  
compounds which increase an ARP polypeptide activity are  
considered as potential ARP polypeptide agonists and

further as potential therapeutic agents for the treatment of neoplastic conditions of the prostate. Each of these classes of compounds is encompassed by the term ARP regulatory agent as defined herein.

5                Within the biological arts, the term "about"  
when used in reference to a particular activity or  
measurement is intended to refer to the referenced  
activity or measurement as being within a range of values  
encompassing the referenced value and within accepted  
10 standards of a credible assay within the art, or within  
accepted statistical variance of a credible assay within  
the art.

A reaction system for identifying a compound that inhibits or enhances an ARP polypeptide activity can be performed using essentially any source of ARP polypeptide activity. Such sources include, for example, a prostate cell sample, lysate or fractionated portion thereof; a bodily fluid such as blood, serum or urine from an individual with a prostate neoplastic condition; a recombinant cell or soluble recombinant source, and an *in vitro* translated source. The ARP polypeptide source is combined with an agent that functions to measure an activity associated with an ARP polypeptide as described above and incubated in the presence or absence of a test inhibitory compound. The amount of product that corresponds to an ARP polypeptide activity that is formed in the presence of the test compound is compared with that in the absence of the test compound. Those test compounds which inhibit product formation are considered to be ARP polypeptide inhibitors. For example, a test compound can inhibit product formation by at least 50%,

80%, 90%, 95%, 99%, 99.5% or 99.9%. Similarly, those compounds which increase product formation are considered to be ARP polypeptide enhancers or activators. For example, a test compound can increase product formation  
5 by at least two-fold, five-fold, ten-fold, 100-fold, 200-fold or 1000-fold. ARP polypeptide inhibitors and activators can then be subjected to further *in vitro* or *in vivo* testing to confirm that they inhibit an ARP polypeptide activity in cellular and animal models.

10            Suitable test compounds for the inhibition or enhancement assays can be any substance, molecule, compound, mixture of molecules or compounds, or any other composition which is suspected of being capable of inhibiting an ARP polypeptide activity *in vivo* or *in*  
15 *vitro*. The test compounds can be macromolecules, such as biological polymers, including proteins, polysaccharides and nucleic acid molecules. Sources of test compounds which can be screened for ARP polypeptide inhibitory activity include, for example, libraries of peptides,  
20 polypeptides, DNA, RNA and small organic compounds. The test compounds can be selected randomly and tested by the screening methods of the present invention. Test compounds are administered to the reaction system at a concentration in the range from about 1 pM to 1 mM.

25            Methods for producing pluralities of compounds to use in screening for compounds that modulate the activity of an ARP polypeptide, including chemical or biological molecules that are inhibitors or enhancers of an ARP activity such as simple or complex organic  
30 molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins,

lipoproteins, nucleic acid molecules, antibodies, and the like, are well known in the art and are described, for example, in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et al., Med. Res. Rev. 15:481-496 (1995); and the like. Libraries containing large numbers of natural and synthetic compounds also can be obtained from commercial sources. Combinatorial libraries of molecules can be prepared using well known combinatorial chemistry methods (Gordon et al., J. Med. Chem. 37: 1233-1251 (1994); Gordon et al., J. Med. Chem. 37: 1385-1401 (1994); Gordon et al., Acc. Chem. Res. 29:144-154 (1996); Wilson and Czarnik, eds., Combinatorial Chemistry: Synthesis and Application, John Wiley & Sons, New York (1997)).

Therefore, the invention provides a method of identifying a compound that inhibits or enhances an ARP polypeptide activity where the sample further consists of a prostate cell lysate, a recombinant cell lysate expressing an ARP polypeptide, an *in vitro* translation lysate containing an ARP mRNA, a fraction of a prostate cell lysate, a fraction of a recombinant cell lysate expressing an ARP polypeptide, a fractionated sample of an *in vitro* translation lysate containing an ARP mRNA or an isolated ARP polypeptide. The method can be performed in single or multiple sample format.

In another embodiment, polypeptides of the invention can be used as vaccines to prophylactically treat individuals for the occurrence of a prostate neoplastic condition or pathology. Such vaccines can be

used to induce B or T cell immune responses or both aspects of the individuals endogenous immune mechanisms. The mode of administration and formulations to induce either or both of these immune responses are well known to those skilled in the art. For example, polypeptides can be administered in many possible formulations, including pharmaceutically acceptable mediums. They can be administered alone or, for example, in the case of a peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can include or be administered in conjunction with an adjuvant, various of which are known to those skilled in the art. After initial immunization with the vaccine, further boosters can be provided if desired. Therefore, the vaccines are administered by conventional methods in dosages which are sufficient to elicit an immunological response, which can be easily determined by those skilled in the art. Alternatively, the vaccines can contain anti-idiotypic antibodies which are internal images of polypeptides of the invention. Methods of making, selecting and administering such anti-idiotypic vaccines are well known in the art. See, for example, Eichmann, et al., CRC Critical Reviews in Immunology 7:193-227 (1987). In addition, the vaccines can contain an ARP nucleic acid molecule. Methods for using nucleic acid molecules such as DNA as vaccines are well known to those skilled in the art (see, for example, Donnelly et al. (Ann. Rev. Immunol. 15:617-648 (1997))); Felgner et al. (U.S. Patent No. 5,580,859, issued December 3, 1996); Felgner (U.S. Patent No. 5,703,055, issued December 30, 1997); and Carson et al. (U.S. Patent No. 5,679,647, issued October 21, 1997)).

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The invention additionally provides a method of treating or reducing the severity of a prostate neoplastic condition. The method is practiced by administering to an individual having a prostate

5 neoplastic condition or other prostatic pathology an ARP1, ARP2, ARP3, ARP4 or ARP5 regulatory agent. A "regulatory agent" means an agent that inhibits or enhances a biological activity of the specified ARP polypeptide. Such an ARP regulatory agent can effect the

10 amount of ARP polypeptide produced or can inhibit or enhance activity without effecting the amount of polypeptide. Such an ARP regulatory agent can be, for example, a dominant negative form of ARP1, ARP2, ARP3, Arp4 or Arp5; a ARP3, ARP4 or ARP selective binding

15 agent, or an antisense molecule. One skilled in the art understands that such an ARP1, ARP2, ARP3, ARP4 or ARP5 regulatory agent can be an agent that selectively regulates a biological activity of the specified ARP polypeptide or, alternatively, can be a non-selective

20 agent that, in addition to regulating a biological activity of the specified polypeptide, also regulates the activity of one or more polypeptides.

A ARP regulatory agent can cause a two-fold, five-fold, ten-fold, 20-fold, 100-fold or more reduction

25 in the amount or activity of an ARP polypeptide. As another example, a regulatory agent can cause a two-fold, five-fold, ten-fold, 20-fold, 100-fold or more increase in the amount or activity of an ARP polypeptide or nucleic acid. ARP regulatory agents include ARP nucleic

30 acid molecules, including antisense nucleic acid molecules, and other non-ARP nucleic acid molecules; binding agents including antibodies, and compounds

identified by the methods described herein. Such regulatory agents can be useful as therapeutics for treating or reducing the severity of an individual with a prostate neoplastic condition or pathology.

5 One type of ARP regulatory agent is an inhibitor, means an agent effecting a decrease in the extent, amount or rate of ARP polypeptide expression or activity. An example of an ARP inhibitor is an ARP antisense nucleic acid molecule or a transcriptional  
10 inhibitor that binds to an ARP 5' promoter/regulatory region.

The term inhibitory amount means the amount of an inhibitor necessary to effect a reduction in the extent, amount or rate of ARP polypeptide. For example,  
15 an inhibitory amount of inhibitor can cause a two-fold, five-fold, ten-fold, 20-fold, 100-fold or more reduction in the amount or activity of an ARP polypeptide of the invention.

Such inhibitors can be produced using methods  
20 which are generally known in the art, and include the use of a purified ARP polypeptide to produce antibodies or to screen libraries of compounds, as described previously, for those which specifically bind a corresponding ARP polypeptide. For example, in one aspect, antibodies  
25 which are selective for an ARP polypeptide of the invention can be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a cytotoxic or cytostatic agent to neoplastic prostate cells. Such agents can be, for example,  
30 radioisotopes. The antibodies can be generated using

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methods that are well known in the art and include, for example, polyclonal, monoclonal, chimeric, humanized single chain, Fab fragments, and fragments produced by a Fab expression library.

5           In another embodiment of the invention, ARP polynucleotides, or any fragment thereof, or antisense molecules, can be used as an ARP regulatory agent in a method of the invention. In one aspect, antisense molecules to an ARP encoding nucleic acid molecules can  
10 be used to block the transcription or translation of the corresponding mRNA. Specifically, cells can be transformed with sequences complementary to a nucleic acid molecule of the invention. Such methods are well known in the art, and sense or antisense oligonucleotides  
15 or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding ARP polypeptides or nucleic acids. Thus, antisense molecules may be used to modulate an ARP activity, or to achieve regulation of an ARP gene  
20 function.

Expression vectors derived from retroviruses, adenovirus, adeno-associated virus (AAV), herpes or vaccinia viruses, or from various bacterial plasmids can be used for delivery of antisense nucleotide sequences to  
25 the prostate cell population. The viral vector selected should be able to infect the tumor cells and be safe to the host and cause minimal cell transformation. Retroviral vectors and adenoviruses offer an efficient, useful, and presently the best-characterized means of  
30 introducing and expressing foreign genes efficiently in mammalian cells. These vectors are well known in the art

Ribozymes, which are enzymatic RNA molecules, can also be used to catalyze the specific cleavage of an ARP mRNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target ARP RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within any potential RNA target are identified by scanning an ARP RNA for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for secondary structural features which can render the oligonucleotide inoperable. The suitability of candidate targets can also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Antisense molecules and ribozymes of the invention can be prepared by any method known in the art for the synthesis of nucleic acid molecules.

In another embodiment, an ARP promoter and regulatory region can be used for constructing vectors for prostate cancer gene therapy. The promoter and regulatory region can be fused to a therapeutic gene for prostate specific expression. This method can include the addition of one or more enhancer elements which amplify expression of the heterologous therapeutic gene without compromising tissue specificity. Methods for identifying a gene promoter and regulatory region are well known to those skilled in the art, for example, by selecting an appropriate primer from the 5' end of the coding sequence and isolating the promoter and regulatory region from genomic DNA.

Examples of therapeutic genes that are candidates for prostate gene therapy utilizing an ARP promoter include suicide genes. The expression of suicide genes produces a protein or agent that directly or indirectly inhibits neoplastic prostate cell growth or promotes neoplastic prostate cell death. Suicide genes include genes encoding enzymes, oncogenes, tumor suppressor genes, genes encoding toxins, genes encoding cytokines, or a gene encoding oncostatin. The therapeutic gene can be expressed using the vectors described previously for antisense expression.

In accordance with another embodiment of the present invention, there are provided diagnostic systems, for example, in kit form. Such a diagnostic system contains at least one nucleic acid molecule or antibody of the invention in a suitable packaging material. The diagnostic kits containing nucleic acid molecules are derived from ARP nucleic acid molecules described herein.

A diagnostic system of the invention can be useful for assaying for the presence or absence of an ARP nucleic acid molecule in either genomic DNA or mRNA.

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A suitable diagnostic system includes at least  
5 one ARP nucleic acid molecule or antibody, as a  
separately packaged chemical reagent(s) in an amount  
sufficient for at least one assay. For a diagnostic kit  
containing a nucleic acid molecule of the invention, the  
kit will generally contain two or more nucleic acid  
10 molecules. When the diagnostic kit is to be used in PCR,  
the kit can further contain at least two oligonucleotides  
that can serve as primers for PCR. Those of skill in the  
art can readily incorporate nucleic acid molecules  
antibodies of the invention into kit form in combination  
15 with appropriate buffers and solutions for the practice  
of the invention methods as described herein. A kit  
containing an ARP polypeptide-specific antibody can  
contain a reaction cocktail that provides the proper  
conditions for performing an assay, for example, an ELISA  
20 or other immunoassay, for determining the level of  
expression of a corresponding ARP polypeptide in a  
specimen, and can contain control samples that contain  
known amounts of a corresponding ARP polypeptide and, if  
desired, a second antibody selective for the  
25 corresponding anti-ARP antibody.

The contents of the kit of the invention, for  
example, ARP nucleic acid molecules or antibodies, are  
contained in packaging material, which can provide a  
sterile, contaminant-free environment. In addition, the  
30 packaging material contains instructions indicating how  
the materials within the kit can be employed both to

detect the presence or absence of a particular nucleic acid sequence or polypeptide of the invention or to diagnose the presence of, or a predisposition for a condition associated with the presence or absence of a nucleic acid sequence or polypeptide of the invention such as prostate cancer. The instructions for use typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

#### **EXAMPLE I**

##### **Isolation of ARP cDNA**

This example describes the isolation of ARP1, ARP2, ARP3, ARP4 AND ARP5 cDNAs.

To identify genes transcriptionally regulated by androgens, microarrays containing prostate derived

cdNAs were screened using RNA from a prostate cell line. Those RNAs showing increased expression levels in response to androgen stimulation were identified and characterized further. Specifically, the microarrays were constructed from a non-redundant set of 1500 prostate-derived cDNA clones identified from the Prostate Expression Database, a public sequence repository of expressed sequence tag (EST) data derived from human prostate cDNA libraries (Hawkins et al., Nucleic Acids Res. 27: 204-208 (1999)). The 1500 prostate cDNA clones were selected from the Prostate Expression Database by randomly selecting the first 1500 non-redundant clones that were in the database. The 1500 prostate cDNA clones were randomly selected from the database using the criteria of taking the first 1500 non-redundant clones in the database and available based on their prior characterization as prostate-derived. Individual clones from the library were obtained and inserts were amplified using primers corresponding to priming sites flanking the insert cloning site of the pSport1 plasmid (Life Technologies, Germantown, MD) as previously described by Nelson et al. (Genomics 47:12-25 (1998)). PCR products were purified through Sephadryl S500 (Pharmacia, Kalamazoo, MI), mixed 1:1 with denaturing Reagent D (Amersham, Piscataway, NJ), and spotted in duplicate onto coated Type IV glass microscope slides (Amersham, Piscataway, NJ) using a Molecular Dynamics (Sunnyvale, CA) Gen II robotic spotting tool. After spotting, the slides were air-dried and UV-crosslinked with 500mJ of energy.

To identify genes transcriptionally regulated by androgens, the microarrays of prostate derived cdNAs



5 TRIzol (Life Technologies, Germantown, MD) according to  
the manufacturer's directions. The integrity of the RNA  
preparation was checked on a standard formaldehyde  
agarose gel. Fifty  $\mu\text{g}$  of the total RNA was digested with  
1  $\mu\text{l}$  of RNase-free DNase (Promega, Madison, WI) ( $1\mu/\mu\text{l}$ )  
10 in 1X first strand cDNA synthesis buffer (Gibco-BRL,  
Germantown, MD) at 37°C for 30 minutes. The reaction mix  
was then extracted with Phenol/chloroform (1:1) and RNA  
was precipitated with ethanol. The mRNA was isolated  
from the DNA-free total RNA using a Dynabeads mRNA  
15 purification kit (Dynal, Lake Success, NY).

Fluorescence-labeled probes were constructed from the above-isolated mRNA as follows. Briefly, 1  $\mu$ g polyA<sup>+</sup> RNA or 30  $\mu$ g total RNA in a reaction volume of 20  $\mu$ l containing 1  $\mu$ l anchored oligo-dT primer (Amersham, Piscataway, NJ), 0.05 mM Cy3-dCTP (Amersham, Piscataway, NJ), 0.05 mM dCTP, 0.1 mM each dGTP, dATP, dTTP, and 200 U Superscript II reverse transcriptase (Life Technologies, Germantown, MD) were incubated at 42°C for 90 minutes followed by heating to 94°C for 3 minutes. Unlabeled RNA was hydrolyzed by the addition of 1  $\mu$ l of 5M NaOH and heating to 37°C for 10 minutes. One  $\mu$ l of 5M HCl and 5  $\mu$ l of 1 M Tris-HCl (pH 7.5) were added to neutralize the base. Unincorporated nucleotides and salts were removed by chromatography (Qiagen, Valencia, CA), and the cDNA was eluted in 30  $\mu$ l distilled water.

Microarray hybridization was performed as follows. One  $\mu\text{g}$  of dA/dT 12-18 (Pharmacia, Kalamazoo, MI) and 1  $\mu\text{g}$  of Cot1 DNA (Life Technologies, Germantown, MD) were added to the probe, heat denatured at  $94^{\circ}\text{C}$  for 5 minutes, combined with an equal volume of 2X microarray hybridization solution (Amersham, Piscataway, NJ) and placed onto the microarray slide with a coverslip. Hybridization was carried out in a humid chamber at  $52^{\circ}\text{C}$  for 16 hours. The slides were washed once with 1X SSC, 10 0.2% SDS at room temperature for 5 minutes, then twice washed with 0.1X SSC, 0.2% SDS at room temperature for 10 minutes. After washing, the slide was rinsed in distilled water to remove trace salts and dried.

Analysis of the microarray slides to identify 15 androgen-regulated prostate genes was performed as follows. Fluorescence intensities of the immobilized targets were measured using a laser confocal microscope (Molecular Dynamics, Sunnyvale, CA). Intensity data were integrated at a pixel resolution of 10 micrometers using 20 approximately 20 pixels per spot, and recorded at 16 bits. Local background hybridization signals were subtracted prior to comparing spot intensities and determining expression ratios. For each experiment, each cDNA was represented twice on each slide, and the 25 experiments were performed in duplicate producing four data points per cDNA clone per hybridization probe. Intensity ratios for each cDNA clone hybridized with probes derived from androgen-stimulated LNCaP and androgen-starved LNCaP were calculated (stimulated 30 intensity/starved intensity). A gene expression level change was treated as significantly different between the two conditions if all four replicate spots for a given

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cdNA demonstrated a ratio greater than 2 or less than  $\frac{1}{2}$  and the signal intensity was greater than 2 standard deviations above the image background. It had been determined previously that expression ratios less than 5 2-fold are not reproducible in this system.

Of a total of 1500 distinct cDNAs represented on the microarray, several were identified as giving a differential signal with the androgen-stimulated probe as compared to the non-stimulated probe.

10 Additional cDNA clones were obtained from screening human prostate 5' stretch cDNA (ClonTech, Inc.) With the original cDNA clones and sequences. RACE was performed using Marathon Ready human prostate cDNAs from ClonTech and using cDNA prepared from androgen-stimulated  
15 LNCaP cells with the Marathon cDNA amplification kit (ClonTech Inc.) according to manufacturer's protocol.

As shown in Figure 1, the ARP1 cDNA has a nucleotide sequence of 1026 nucleotides. As shown in Figure 2, ARP2 cDNA contains 4509 nucleotides predicted to encode a polypeptide 252 amino acids long. As shown in Figure 3, ARP3 cDNA contains 2213 nucleotides predicted to encode a polypeptide 538 amino acids long.

The ARP4 cDNA was isolated as described above. RACE was performed using the following primers: RC55  
25 (5'-TGAGGTATCCCAGAGCAAACACAAAGCAG-3'; SEQ ID NO: 10 ) and RC202 (5'-TCAGTTCTTCATCCTTCCGAAACATCCC-3'; SEQ ID NO: 11). RACE reactions were performed according to the standard ClonTech protocol, and the resulting nucleic acids sequenced by standard methods. Two related cDNAs

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